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EVALUATION OF THE POTENTIAL OF INHALED CHLOROPENTAFLUOROBENZENE TO INDUCE TOXICITY IN F-344 RATS AND B6C3F1 MICE AND SISTER CHROMATID EXCHANGES AND MICRONUCLEI FORMATION IN B6C3F1 MICE

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TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-89-037

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

MICHAEL B. BALLINGER, Lt Col, USAF, BSC
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Ten Fischer 344 rats and six B6C3F1 mice of each sex were exposed to air, 0.25, 0.80, or 2.50 mg chloropentafluorobenzene (CPFB)/L of air for 3 weeks, excluding weekends. Exposure to 2.50 mg/L caused a reduction in the growth rate of rats but did not affect the growth rate of mice. Following the exposure there was reduced SGOT activity in the blood serum of exposed rats and a dose related increase in liver weights. Increased liver weights were observed in mice as well; the response in the female groups was clearly dose dependent. Histologically the livers of both rats and mice presented single cell necrosis. In exposed mice hepatocytes exhibited mild hepatocytomegaly with increased granular eosinophilic cytoplasm. In evaluations for its potential to induce chromosomal damage following this exposure regimen, CPFB did not alter the rate of bone marrow cellular proliferation. Assessment of the micronucleated polychromatic erythrocytes and normochromatic erythrocyte populations during the inhalation exposures indicated a general absence of genotoxic activity.

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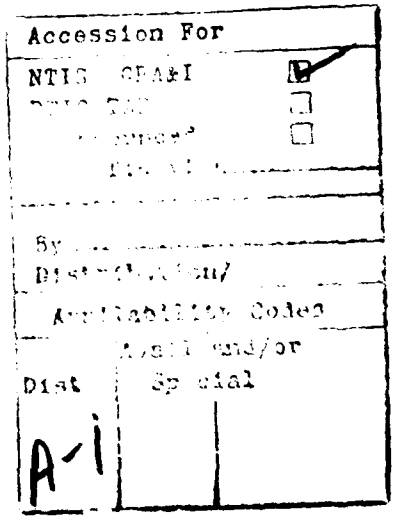
PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted in the Toxic Hazards Research Unit, NSI Technology Services Corporation – Environmental Sciences. This document serves as a final report on selected toxicity studies of chloropentafluorobenzene (CPFB). The research described in this report began in June 1988 and was completed in June 1989. It was performed under U.S. Air Force Contract No. F33615-85-C-0532. Melvin E. Andersen, Ph.D., served as a Contract Technical Monitor for the U.S. Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Uses of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #85-23, 1985, and the Animal Welfare Act of 1966, as amended.



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ABBREVIATIONS

AGT	Average generation time
BUN	Blood urea nitrogen
CPFB	Chloropentafluorobenzene
dL	Deciliter
F-344	Fischer 344 (rats)
fL	Femtoliter
g	Gram
h	Hour
kg	Kilogram
L	Liter
MCV	Mean corpuscular volume
mg	Milligram
MI	Mitotic index
min	Minute
mL	Milliliter
mm	Millimeter
MN	Micronuclei
MN-NCE	Micronuclei in normochromatic erythrocytes
MN-PCE	Micronuclei in polychromatic erythrocytes
N	Number of animals
NCE	Normochromatic erythrocytes
nm	Nanometer
p	Probability
PCE	Polychromatic erythrocytes
pg	Picogram

ABBREVIATIONS (continued)

ppm	Parts per million
RBC	Red blood cells
RI	Replication index
SCE	Sister chromatid exchange
SEM	Standard error mean
SGOT	Serum glutamic oxaloacetic transaminase
THRU	Toxic Hazards Research Unit

SECTION 1

INTRODUCTION

Chloropentafluorobenzene (CPFB) has been selected as a candidate material for use as a CW simulant for training purposes. Preliminary screening has indicated that CPFB provides good detectability for biological monitoring, desirable partitioning in biological tissues, acceptable physical properties, and relative biological inertness (Jepson et al., 1985).

The primary irritation hazard, sensitization potential, and acute inhalation toxicity of CPFB have been evaluated at the Toxic Hazards Research Unit (THRU) (Kinkead et al., 1987). CPFB demonstrated no potential for skin sensitization in guinea pigs; however, it did produce mild skin and conjunctival irritation in rabbits. Short-term exposure to CPFB vapor posed no serious hazard by the inhalation route as all rats survived a 4-h exposure to 4.84 mg/L. It was necessary to evaluate the effects of repeated inhalation exposure to this material because it has been anticipated that under the conditions of intended use individuals may be exposed to this simulant on a short-term repeated or, in the case of instructors, reoccurring basis.

Although the genotoxic potential of CPFB has been investigated in several studies (Tu et al., 1986; Steele, 1987), the results have been equivocal. In addition, the relevancy of the high concentrations of CPFB used made the results difficult to assess. B6C3F1 mice were, therefore, included in this study to investigate the potential of CPFB to cause genotoxic and cytotoxic damage *in vivo*. Induction of sister chromatid exchange (SCE) in bone marrow metaphase cells, micronuclei in polychromatic erythrocytes (MN-PCE), and the inhibition of bone marrow cellular proliferation were measured. The assessment of the genotoxic and cytotoxic damage in these animals was performed under subcontract. The required tissue samples were collected from animals within the THRU facilities.

The rat was selected as the test species for the short-term repeated inhalation portion of this study. The species and number of rats per group were selected to conform with the U.S. Environmental Protection Agency's Health Effects Test Guidelines (1982) and to allow for statistical evaluation of the results. Existing alternative methods to animal testing were considered inadequate for this study.

The mouse was selected as the test species for the cytogenetic studies (genotoxicity section) because the lifetime of normochromatic erythrocytes (NCE) in the mouse permits analysis of the damage inducted in bone marrow over the duration of the study while the 1 to 2 day lifespan of polychromatic erythrocytes (PCE) permitted assessment of acute damage.

SECTION 2

MATERIALS AND METHODS

Animals

Upon receipt from Charles River Breeding Labs (Raleigh, NC), male and female Fischer 344 (F-344) rats, 9-11 weeks of age, were found to be in acceptable health. The animals were randomized and group housed (two to three per cage) in clear plastic cages with wood chip bedding prior to the study. The rats were housed individually and assigned to specific cage locations at the beginning of the inhalation studies.

B6C3F1 male and female mice, 9-11 weeks of age, were purchased from Harlan Sprague-Dawley (Indianapolis, IN). After quality control testing, they were found to be in acceptable health. The animals were randomized and group housed (five per cage) in clear plastic cages with wood chip bedding prior to the study. During the study, rats and mice were individually housed in the inhalation chambers.

Water and feed (Purina Formulab #5008, St. Louis, MO) were available *ad libitum*, except during the inhalation exposure period and when the rats were fasted for 10 h prior to sacrifice. The light/dark cycle was set at 12-h intervals.

Test Agent

The CPFB used in this study was purchased from Aldrich Chemical Co. (Milwaukee, WI). The physical properties of CPFB are shown in **Table 1**.

TABLE 1. PHYSICAL PROPERTIES OF CHLOROPENTAFLUOROBENZENE

Chemical Formula	C_6C1F_5
Molecular Weight	202
Boiling Point (°C)	117
Density (g/mL)	1.66
Vapor Pressure (mmHg, 25°C)	14.1

Test Agent Quality Control

The purity of the test material was determined by capillary gas chromatography. A Varian 3700 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with an electron capture detector was used in conjunction with a Hewlett-Packard 3388 computing integrator (Hewlett-

Packard Co , Palo Alto, CA) to measure peak area and record chromatograms. CPFB was diluted in hexane to provide peak areas within the detection limits of the instrument.

Generation and Analysis of Exposure Atmospheres

CPFB vapor was generated by metering air through a glass fritted dispersion tube immersed in a gas washing bottle containing liquid CPFB. The saturated vapor was delivered into the chamber through a stainless steel tube where it was mixed countercurrently with chamber input air. Concentration was controlled by adjusting the volume of air passing through the gas washing bottle.

The chamber atmospheres were analyzed continuously using a Miran 1A infrared analyzer (Foxboro, S. Norwalk, CT). The path length varied for each chamber depending on the target concentration while the wavelength, 11.3 (± 0.1) mm, was used for each instrument.

Exposure Regimen

Ten male and ten female F-344 rats and six male and six female B6C3F1 mice, 9-11 weeks of age, were placed in four 690-L inhalation chambers and exposed for 6 h daily, excluding weekends (15 exposure days over a three-week period) to air only, 0.25, 0.80, and 2.50 mg CPFB/L, respectively. They were housed individually and assigned to specific exposure cage locations. The exposure cages were rotated clockwise (moving one position) within the inhalation chambers each exposure day. Test and control groups were sacrificed on the day following the 15th exposure.

Toxicity Assessments

Records were maintained of body weights, signs of toxicity, and mortality. At sacrifice, gross pathology was performed on all animals and tissues (**Tables 2 and 3**) were harvested for histopathologic examination. Wet tissue weights were determined on adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes, and thymus from the exposed rats. From exposed mice, wet tissue weights were determined on adrenals, brain, epididymis, heart, kidneys, liver, lungs, ovaries, testes, spleen, uterus, and vagina. Tissues for histopathologic examination were fixed in 10% neutral buffered formalin, trimmed, and further processed via routine methods for HE-stained, paraffin-embedded sections (Luna, 1968). Additionally, blood was drawn from the rats for hematology (**Table 4**) and clinical chemistry (**Table 5**) assays. Erythrocytes were enumerated on a Coulter Counter (Coulter Electronics, Hialeah, FL) and sera for clinical chemistry evaluation were assayed on an Ektachem 700 XR (Eastman Kodak, Rochester, NY). Selected hematological parameters and absolute leukocyte differentials were determined according to established procedures.

TABLE 2. TISSUES HARVESTED FROM CONTROL AND CPFB-EXPOSED F-344 RATS FOR HISTOPATHOLOGIC EXAMINATION

Gross lesions	Mandibular lymph nodes	Uterus
Thyroid/parathyroid	Mesenteric lymph nodes	Esophagus
Lungs	Eye	Stomach
Trachea	Preputial glands	Colon
Heart	Thymus	Rectum
Liver	Brain	Sternum
Spleen	Kidneys	Sciatic nerve
Duodenum	Adrenals	Skeletal muscle
Jejunum	Pancreas	Pituitary glands
Ileum	Gonads	Skin
Urinary bladder	Nasal turbinates (3 sections)	Prostate
Mammary glands	Salivary glands	Cecum
Diaphragm	Epididymis	
Larynx	Conjunctivae	

TABLE 3. TISSUES HARVESTED FROM CONTROL AND CPFB-EXPOSED B6C3F1 MICE FOR HISTOPATHOLOGIC EXAMINATION

Gross lesions	Eye	Nasal turbinates (4 sections)
Thyroid/parathyroid	Diaphragm	Uterus
Lungs	Pituitary glands	Esophagus
Trachea	Larynx	Stomach
Heart	Epididymis	Colon
Liver	Salivary glands	Rectum
Spleen	Mammary gland	Sternum
Duodenum	Thymus	Sciatic nerve
Jejunum	Brain	Skeletal muscle
Ileum	Kidneys	Preputial glands
Urinary bladder	Adrenals	Gall bladder
Mandibular lymph nodes	Pancreas	Prostate
Mesenteric lymph nodes	Gonads	Cecum
		Skin

TABLE 4. ASSAYS PERFORMED ON WHOLE BLOOD FROM CONTROL AND CPFB-EXPOSED F-344 RATS

Hematocrit
Hemoglobin
Red blood cell count
Total and differential leucocyte count

TABLE 5. SERUM CHEMISTRY ASSESSMENTS OF CONTROL AND CPFB-EXPOSED F-344 RATS

Creatinine	Chloride
Calcium	Phosphorus
Total protein	Alkaline phosphatase
Blood urea nitrogen	Serum glutamic-pyruvic transaminase
Serum glutamic-oxaloacetic transaminase	Lactate dehydrogenase

SECTION 3

GENOTOXICITY ASSESSMENT

Treatment Regimen

On day 0 and days 9, 16, and 21 of exposure, two peripheral blood smears were prepared for micronuclei (MN) analysis by using blood obtained from the clipped tail of each mouse. The last sampling took place at the time of BrdUrd tablet (Boehringer Mannheim, Indianapolis, IN) implantation. The smears were fixed in absolute methanol (approximately 5 min). Approximately 1 h after the completion of the last exposure, a 50 mg BrdUrd tablet coated partially (~70%) with paraffin (McFee et al., 1984) was implanted subcutaneously into each lightly anesthetized (Metofane®, Pittman-Moore, Inc., Washington Crossing, NJ) animal. In an attempt to score both chromosome aberration (in first generation metaphase cells) and SCE (in second generation metaphase cells) in the same animal, mice were sacrificed from 20 to 21 h post-BrdUrd tablet implantation. Approximately 2 h prior to sacrifice, each animal was injected intraperitoneally with 2 mg/kg colchicine (Eli Lilly, Indianapolis, IN). Euthanasia was accomplished by CO_2 asphyxiation and the bone marrow removed by flushing both femurs with phosphate-buffered saline (pH 7.4). The aspirated bone marrow was incubated in 0.075 M KCl for 25 min at 37° C, fixed first with absolute methanol (10 min) and then with ice-cold 3:1 methanol:glacial acetic acid. After fixation, the bone marrow material was kept cold until slide preparation.

Each bone marrow sample was washed twice in fixative and coded, flame-dried slides were prepared. Slides were differentially stained using a modified (Tice et al., 1978) fluorescence-plus-Giemsa technique (Goto et al., 1978). To examine the bone marrow proliferation kinetics for each mouse, 100 randomly selected metaphase cells (>20 chromosomes) were scored at x 250 magnification for replicative history (i.e., the number of S phases completed since the time of BrdUrd tablet implantation) (Tice et al., 1976). Metaphase cells with intermediate differential staining patterns denoting the incorporation of BrdUrd after S phase had been initiated were scored as having completed that S phase. Twenty-five second generation metaphase cells per animal (four mice per exposure group) were scored at x 1000 magnification for SCE, with selection being based on good

chromatid differential staining, a lack of overlapping chromosomes, and chromosome number (40 ± 3 chromosomes). All scoring was conducted without knowledge of the exposure concentration.

For MN evaluation, coded peripheral blood smears were stained with the DNA-specific stain, acridine orange, as described in Tice et al. (1987). Each slide was scanned at ×800 to ×1000 magnification using epi-illuminated fluorescence microscopy (450-490 nm excitation, 520 nm emission) for the number of MN-PCE among 1000 PCE, the number of micronuclei in normochromatic erthrocytes (MN-NCE) among 1000 NCE, and the percentage of PCE in 1000 erythrocytes. At the same time, information on the number of erythrocytes with 0, 1, 2, etc. MN was retained.

To evaluate scorer reliability, coded slides obtained from male B6C3F1 mice exposed to 7,12 dimethylbenz(a)anthracene (DMBA) (2.5 mg/kg, delivered in corn oil via intraperitoneal injection) 42 h prior to bone marrow sampling were included among the CPFB bone marrow slides. In a similar fashion, coded peripheral blood smears from male C57Bl/6 mice exposed to benzene (100 ppm, 6 h/day for 20 weeks [5 days per week]) were included among the peripheral blood smears obtained from CPFB-exposed mice.

Statistical Analysis

Body weight means and associated standard errors were calculated or assessed for statistical differences according to the repeated multivariate analysis of variance with Scheffe pairwise comparisons (Barcikowski, 1983). A two-factorial analysis of variance with multivariate comparisons (Barcikowski, 1983) was used to analyze the hematology, clinical chemistry, and organ weight data. The histopathology data were analyzed using the following nonparametric tests: Fischer's Exact Test and Yates' Corrected Chi-square (Zar, 1974). A probability of 0.05 inferred a significant change from controls.

The genotoxicity data were first analyzed using a Bloon-Forsythe two-way ANOVA to determine whether a significant sex difference in response occurred. If not, the subsequent analysis was based on pooled data; if significant, each sex was evaluated separately. A one-tailed trend test (Margolin et al., 1986) was used to determine if a treatment-related increase occurred for SCE or MN erythrocyte data. For average generation time (AGT), mitotic index (MI), and %PCE data, a two tailed trend test was used to determine if a treatment-related effect occurred. For SCE, AGT, MI, and %PCE data, the ability of the treatment to affect the group mean values was analyzed by the trend test using individual animal responses. For MN data, the number of MN-PCE or MN-NCE was calculated across animals within each exposure group and analyzed by a one-tailed trend test (Margolin and Risko, 1986).

For statistically significant responses, pairwise comparisons between each exposure group and the corresponding concurrent control group were conducted using the t test or Pearson's Chi-square

test (with the alpha level Bonferroni-corrected for multiple comparisons), as appropriate, to determine the minimal effective dose for each endpoint. For SCE data an additional statistical test, the Dispersion test (Margolin and Shelby, 1985), based on the ratio of the sample variance to the sample mean, was used to evaluate the effect of the exposure to CPFB on the intercellular distribution of SCE within each animal. For the analysis of cellular proliferation kinetics, the proportion of first-, second-, and third-generation metaphase cells in each bone marrow sample was transformed into an AGT (Ivett and Tice, 1982), where AGT is equal to BrdUrd exposure duration/replicative index (RI). The RI is equal to the frequency of first-generation metaphase cells plus two times the frequency of second-generation metaphase cells plus three times the frequency of third-generation metaphase cells plus . . . (Schneider and Lewis, 1981). In addition to the trend test for evaluating the number of micronucleated cells, the proportions of cells with 0, 1, 2, etc. MN were evaluated to determine whether the intercellular distribution of events was significantly affected by the exposure to CPFB.

SECTION 4

RESULTS

Chamber Analysis

The specified nominal concentrations of 0.25, 0.80, and 2.50 mg CPFB/L were maintained during the three-week exposure period. All chamber daily mean concentrations were maintained within ± 10% of the desired concentrations. Mean concentrations for each exposure chamber, along with the high and low daily mean concentrations, are provided in Table 6.

TABLE 6. ANALYSIS OF CPFB CONCENTRATIONS INHALED BY RATS AND MICE FOR 21 DAYS

Target Concentration	0.25 mg/L	0.80 mg/L	2.50 mg/L
Mean concentration			
Rats N = 16	0.25	0.79	2.52
Mice N = 15	0.25	0.77	2.55
Standard error			
Rats	<0.01	0.01	0.02
Mice	<0.01	0.01	0.02
Lowest daily average			
Rats	0.24	0.74	2.40
Mice	0.24	0.73	2.40
Highest daily average			
Rats	0.27	0.83	2.65
Mice	0.26	0.81	2.72

Inhalation Toxicity

A total of 80 F-344 rats and 48 B6C3F1 mice were included in the three-week, 15-day inhalation toxicity study. There were no deaths resulting from the exposures. One female mouse from the 2.5 mg CPFB/L concentration group died during the BrdUrd tablet implant procedure. No signs of toxic stress were noted in any of the animals during the three-week exposure period.

Mean body weights of male and female rats exposed to 2.5 mg CPFB/L were significantly depressed during the last two weeks of the three-week study (**Figure 1**). Mean body weights of the male and female test mice did not differ from their respective control group (**Figure 2**).

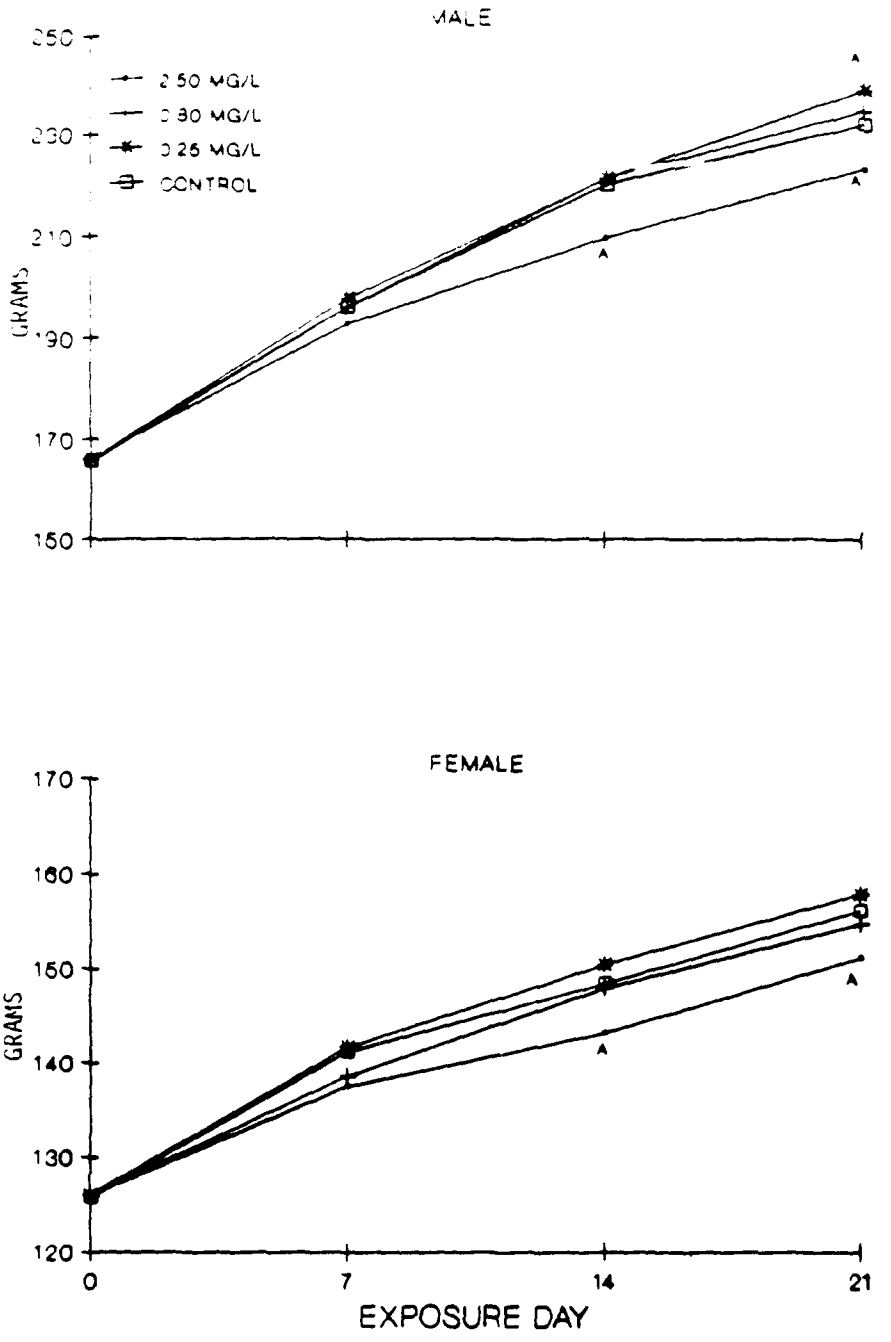

Figure 1. **Effect of 21-Day CPFB Inhalation Exposure on Mean Body Weights of F-344 Male and Female Rats (N = 10).** A = different from control, p 0.01, using repeated multivariate analysis with pairwise comparisons.

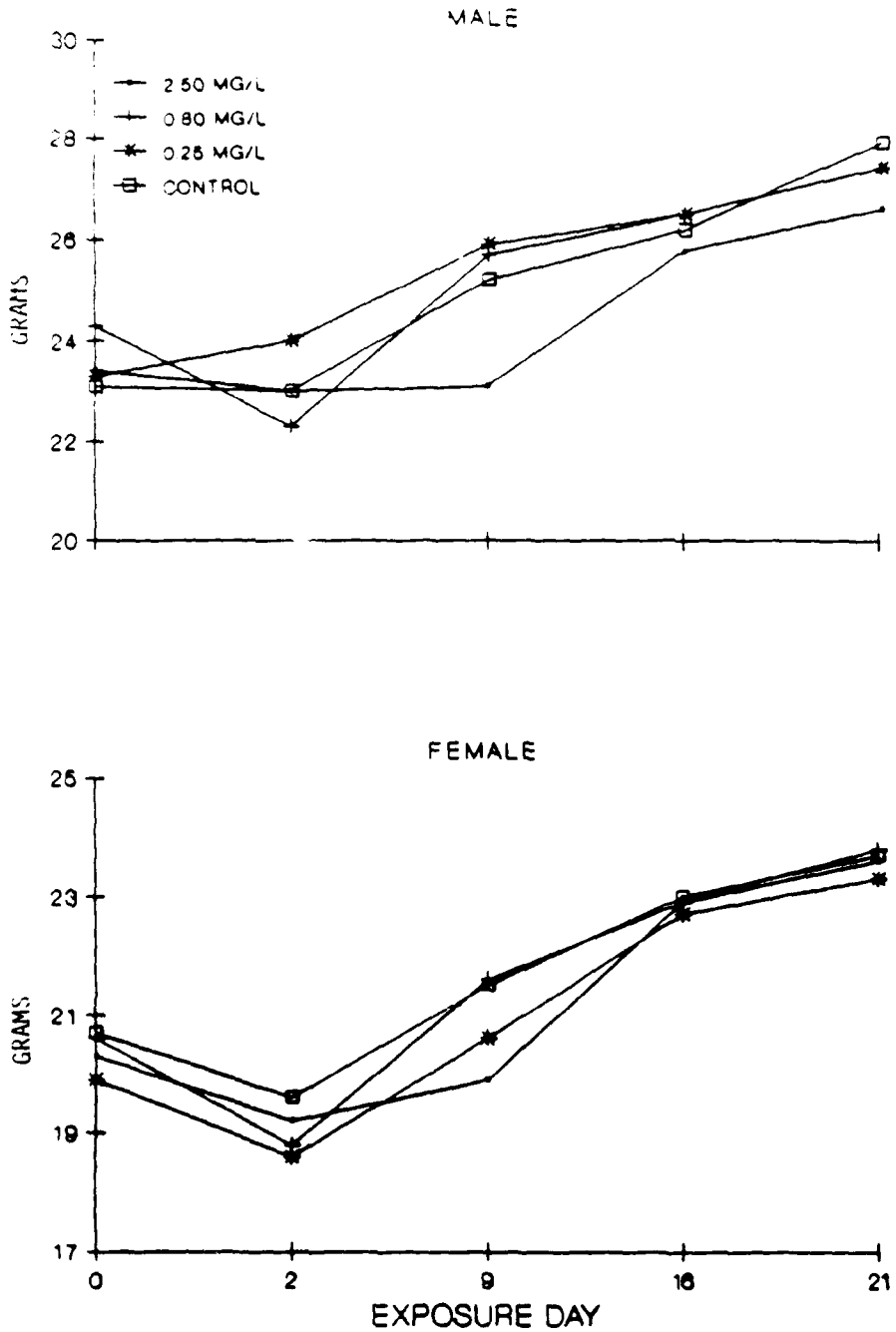

Figure 2. **Effect of 21-Day CPFB Inhalation Exposure on Mean Body Weights of B6C3F1 Male and Female Mice (N = 6).** There were no statistical differences between treatment groups and controls.

Analysis of hematology parameters for male F-344 rats (**Table 7**) revealed no significant differences between test and control groups. Mean corpuscular volume (MCV) was significantly less ($p<0.01$) than the control values for the high-concentration female rat group (**Table 8**), but was still within the laboratory's normal range (52.5 - 72.6 fL/red blood cells [RBC]) and near the value of rats in other groups. All other hematology values were within normal ranges.

TABLE 7. MEAN[a] WHOLE BLOOD PARAMETERS FOR MALE F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

	Control	0.25 mg/L	0.80 mg/L	2.50 mg/L
WBC (x 10^3 cells/mm^3)	7.50 ± 0.29	7.59 ± 0.31[b]	8.06 ± 0.45	7.81 ± 0.28
RBC (x 10^6 cells/mm^3)	8.48 ± 0.26	8.61 ± 0.11	8.73 ± 0.10	8.59 ± 0.14
HGB (g/dL)	16.92 ± 0.24	16.96 ± 0.23	16.95 ± 0.21	16.71 ± 0.21
HCT (%)	45.33 ± 1.33	46.72 ± 0.70	46.83 ± 0.71	45.72 ± 0.83
MCV (fL)	53.40 ± 0.25	53.98 ± 0.35	53.59 ± 0.30	53.16 ± 0.25
MCH (pg)	20.07 ± 0.49	19.69 ± 0.18	19.42 ± 0.12	19.48 ± 0.14
MCHC (%)	37.52 ± 0.88	36.47 ± 0.31	36.18 ± 0.31	36.61 ± 0.30
Neutrophils (%)	15.00 ± 1.91	16.56 ± 2.58	16.90 ± 1.98	13.50 ± 2.32
Lymphocytes (%)	82.20 ± 2.02	79.00 ± 2.75	79.40 ± 2.31	83.60 ± 2.88
Monocytes (%)	3.13 ± 0.69	3.89 ± 0.79	2.89 ± 0.26	2.89 ± 0.68

[a] Mean ± S.E.M., N = 10 animals unless noted otherwise.
[b] N = 9 animals for all values from the 0.25 mg/L group.

TABLE 8. MEAN[a] WHOLE BLOOD PARAMETERS FOR FEMALE F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

	Control	0.25 mg/L	0.80 mg/L	2.50 mg/L
WBC (x 10^3 cells/mm^3)	6.36 ± 0.41	5.67 ± 0.32	7.01 ± 0.36	5.92 ± 0.49
RBC (x 10^6 cells/mm^3)	8.12 ± 0.06	8.13 ± 0.05	8.22 ± 0.18	8.37 ± 0.15
HGB (g/dL)	16.81 ± 0.16	16.37 ± 0.17	16.70 ± 0.24	16.61 ± 0.32
HCT (%)	49.38 ± 0.74	48.07 ± 0.51	49.26 ± 1.05	48.69 ± 0.94
MCV (fL)	60.78 ± 0.73	59.12 ± 0.37	59.84 ± 0.48	58.12 ± 0.46[b]
MCH (pg)	20.69 ± 0.17	20.14 ± 0.18	20.44 ± 0.73	19.85 ± 0.37
MCHC (%)	34.03 ± 0.25	34.05 ± 0.23	34.09 ± 1.13	34.19 ± 0.73
Neutrophils (%)	11.60 ± 1.01	13.60 ± 2.07	12.10 ± 2.05	15.10 ± 2.40
Lymphocytes (%)	85.90 ± 1.20	83.90 ± 2.50	84.60 ± 1.92	82.10 ± 2.22
Monocytes (%)	2.56 ± 0.53	2.67 ± 0.93	3.20 ± 0.70	2.70 ± 0.42

[a] Mean ± S.E.M., N = 10
[b] Significantly different from control at $p<0.05$ using a two-factorial analysis of variance corrected for multiple comparisons

Blood chemistry data from exposed rats are shown in **Tables 9** and **10**. Blood urea nitrogen (BUN) values from rats exposed to 2.5 mg CPFB/L were significantly reduced ($p<0.05$) from the controls, and total protein values of the same group were significantly increased ($p<0.01$) relative to their respective control groups. Serum glutamic-oxaloacetic transaminase (SGOT) values were highest in control groups and gradually decreased as the CPFB concentration increased. Alkaline phosphatase activities were significantly elevated ($p<0.05$) in female rats in the 2.50 mg CPFB/L exposure group only.

TABLE 9. MEAN[a] VALUES OF SERUM BIOCHEMISTRY PARAMETERS FOR MALE F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

	Control	0.25 mg/L	0.80 mg/L	2.50 mg/L
BUN (mg/dL)	12.9 ± 0.4	12.4 ± 0.5[b]	12.6 ± 0.6	11.8 ± 0.3[c]
Creatinine (mg/dL)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
BUN/creatinine	46.2 ± 1.6	40.8 ± 1.9	40.6 ± 1.8	42.9 ± 2.4
Chloride (mmol/L)	100.0 ± 0.4	99.5 ± 0.4	99.6 ± 0.3	99.6 ± 0.5
Calcium (mg/L)	10.5 ± 0.1	10.6 ± 0.1	10.6 ± 0.1	10.7 ± 0.1
Phosphorus (mg/dL)	9.1 ± 0.3	9.4 ± 0.2	9.1 ± 0.2	9.5 ± 0.2
Total protein (g/dL)	5.8 ± 0.0	5.8 ± 0.1	5.9 ± 0.1	6.0 ± 0.1[d]
AST/SGOT (IU/L)	111.0 ± 3.4	107.4 ± 3.8[d]	100.2 ± 2.6[d]	97.5 ± 3.5[d]
ALT/SGPT (IU/L)	59.9 ± 1.3	60.3 ± 3.1	59.2 ± 2.3	55.7 ± 1.9
Alk. Phos. (U/L)	201.5 ± 10.6	223.9 ± 7.7	200.2 ± 7.7	205.6 ± 4.6

[a] Mean ± S.E.M., N = 10 animals unless noted otherwise
[b] N = 9 animals for all values from the 0.25 mg/L group
[c] Significantly different from control at $p<0.05$ using a two-factorial analysis of variance corrected for multiple comparisons
[d] Significantly different from control at $p<0.01$ using a two-factorial analysis of variance corrected for multiple comparisons

TABLE 10. MEAN[a] VALUES OF SERUM BIOCHEMISTRY PARAMETERS FOR FEMALE F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

	Control	0.25 mg/L[b]	0.80 mg/L	2.50 mg/L
BUN (mg/dL)	13.9 ± 0.5	13.4 ± 0.5	12.7 ± 0.3	12.0 ± 0.6[b]
Creatinine (mg/dL)	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0[c]
BUN/creatinine	38.4 ± 3.1	35.8 ± 1.7	35.6 ± 1.5	40.9 ± 2.8
Chloride (mmol/L)	101.9 ± 0.4	102.0 ± 0.5	101.0 ± 0.4	101.8 ± 0.7
Calcium (mg/L)	10.4 ± 0.1	10.4 ± 0.1	10.8 ± 0.1[c]	10.7 ± 0.1
Phosphorus (mg/dL)	8.3 ± 0.3	8.0 ± 0.2	9.0 ± 0.2	8.9 ± 0.3
Total protein (g/dL)	5.7 ± 0.1	5.9 ± 0.1	5.9 ± 0.0	6.0 ± 0.1[c]
Albumin (g/dL)	3.3 ± 0.0	3.5 ± 0.1	3.5 ± 0.0	3.7 ± 0.1[c]
AST/SGOT (IU/L)	116.0 ± 3.2	101.8 ± 2.5[c]	100.9 ± 2.8[c]	107.9 ± 4.5[b]
ALT/SGPT (IU/L)	61.1 ± 1.7	57.8 ± 1.7	64.4 ± 2.4	63.3 ± 2.2
Alk. Phos. (U/L)	175.4 ± 10.5	170.3 ± 7.6	186.4 ± 5.9	213.9 ± 8.2[b]

[a] Mean ± S.E.M., N = 10
[b] Significantly different from control at $p<0.05$ using a two-factorial analysis of variance corrected for multiple comparisons
[c] Significantly different from control at $p<0.01$ using a two-factorial analysis of variance corrected for multiple comparisons

Concentration-related increases in relative liver weights occurred in both sexes of rats (**Tables 11** and **12**). Relative liver weights of the exposed male rats were increased over controls by 8, 13, and 20% in the 0.25, 0.80, and 2.50 mg CPFB/L groups, respectively. The female rats had relative liver weight increases of 3, 8, and 18% in the respective exposure groups. Other significant differences noted, which did not appear to be treatment-related, included an increase in thymus absolute and relative weights in the 0.80 mg CPFB/L exposed male rats and an increase in absolute heart weights of male rats exposed to 0.25 mg CPFB/L. Absolute and relative kidney weights of test male and female rats compared favorably with their respective control group values.

TABLE 11. ORGAN WEIGHTS[a] AND ORGAN TO BODY WEIGHT RATIOS (%) OF MALE F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

	Control	0.25 mg/L	0.80 mg/L	2.50 mg/L
Kidney	1.70 ± 0.03	1.77 ± 0 03	1.76 ± 0.03	1.75 ± 0.05
Ratio[b]	0.78 ± 0.01	0.79 ± 0.01	0.80 ± 0.01	0.82 ± 0.02
Heart	0.83 ± 0.02	0 91 ± 0.02[c]	0.82 ± 0.02	0.78 ± 0.01
Ratio	0.38 ± 0.01	0.41 ± 0.01	0.37 ± 0.01	0.37 ± 0.01
Brain	1.81 ± 0.01	1.82 ± 0.02	1.80 ± 0.02	1.73 ± 0.03
Ratio	0.83 ± 0.01	0 81 ± 0.01	0.82 ± 0.01	0.81 ± 0.01
Liver	6.66 ± 0.15	7.35 ± 0.18[c]	7.57 ± 0.16[d]	7.82 ± 0.25[d]
Ratio	3.04 ± 0 04	3.27 ± 0.06[c]	3.43 ± 0.05[d]	3.66 ± 0.07[d]
Spleen	0.50 ± 0 01	0.49 ± 0.01	0.49 ± 0.01	0.46 ± 0.01
Ratio	0.23 ± 0.00	0.22 ± 0.00	0.22 ± 0.01	0.22 ± <0.01
Thymus	0.29 ± 0.01	0.31 ± 0.02	0.33 ± 0.01[c]	0.30 ± 0.01
Ratio	0.13 ± <0.01	0.14 ± 0.01	0.15 ± 0.01[c]	0.14 ± <0.01
Lungs	1.52 ± 0.11	1.73 ± 0.14	1.86 ± 0.19	1.52 ± 0.12
Ratio	0.70 ± 0.06	0.77 ± 0.06	0.84 ± 0.08	0.71 ± 0.05
Adrenal	0.08 ± <0.01	0.08 ± <0.01	0.08 ± <0.01	0.08 ± <0.01
Ratio	0.04 ± <0.01	0.04 ± <0.01	0.04 ± <0.01	0.04 ± <0.01
Testes	2.83 ± 0.04	2 90 ± 0.04	2.85 ± 0.03	2.81 ± 0 03
Ratio	1.29 ± 0.02	1.29 ± 0.02	1.29 ± 0.02	1.32 ± 0.02
Whole Body[e]	218.90 ± 2.7	224.30 ± 2.6	220.70 ± 2.6	213.00 ± 3.3

[a] Mean ± S E M , N = 10

[b] Organ weight/body weight x 100

[c] Significantly different from control at $p<0.05$ using a two-factorial analysis of variance corrected for multiple comparisons

[d] Significantly different from control at $p<0.01$ using a two-factorial analysis of variance corrected for multiple comparisons

TABLE 12. ORGAN WEIGHTS[a] AND ORGAN TO BODY WEIGHT RATIOS (%) OF FEMALE F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

	Control	0.25 mg/L	0.80 mg/L	2.50 mg/L
Kidney	1.14 ± 0.02	1.17 ± 0.02	1.14 ± 0.02	1.17 ± 0.02
Ratio[b]	0.78 ± 0.02	0.79 ± 0.01	0.78 ± 0.01	0.81 ± 0.01
Heart	0.57 ± 0.02	0.62 ± 0.02	0.58 ± 0.01	0.56 ± 0.01
Ratio	0.39 ± 0.01	0.42 ± 0.02	0.40 ± 0.01	0.39 ± 0.01
Brain	1.68 ± 0.02	1.69 ± 0.02	1.67 ± 0.02	1.64 ± 0.02
Ratio	1.16 ± 0.01	1.14 ± 0.01	1.14 ± 0.01	1.14 ± 0.02
Liver	4.08 ± 0.07	4.28 ± 0.10	4.41 ± 0.07[c]	4.79 ± 0.11[d]
Ratio	2.80 ± 0.04	2.89 ± 0.04	3.01 ± 0.05[c]	3.31 ± 0.06[d]
Spleen	0.38 ± 0.01	0.38 ± 0.01	0.39 ± 0.02	0.36 ± 0.01
Ratio	0.26 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.25 ± 0.01
Thymus	0.27 ± 0.01	0.28 ± 0.02	0.27 ± 0.03	0.28 ± 0.01
Ratio	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.02	0.19 ± 0.01
Lungs	1.18 ± 0.12	1.32 ± 0.09	1.26 ± 0.09	1.24 ± 0.08
Ratio	0.81 ± 0.08	0.89 ± 0.06	0.86 ± 0.06	0.86 ± 0.05
Adrenal	0.07 ± <0.01	0.08 ± <0.01	0.08 ± <0.01	0.07 ± <0.01
Ratio	0.05 ± <0.01	0.05 ± <0.01	0.05 ± <0.01	0.05 ± <0.01
Ovary	0.12 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
Ratio	0.08 ± 0.01	0.08 ± 0.03	0.09 ± 0.01	0.08 ± 0.00
Whole Body[e]	145.70 ± 0.9	148.00 ± 1.7	146.30 ± 1.4	144.80 ± 1.3

[a] Mean ± S.E.M., N = 10

[b] Organ weight/body weight x 100

[c] Significantly different from control at $p<0.05$ using a two-factorial analysis of variance corrected for multiple comparisons

[d] Significantly different from control at $p<0.01$ using a two-factorial analysis of variance corrected for multiple comparisons

[e] Fasted weights

Similar concentration-related increases in relative liver weights occurred in both sexes of mice (**Tables 13** and **14**) with the differences being significant ($p<0.01$) at the high concentration. The relative liver weights of the exposed male mice were increased over controls by 16, 16, and 36% in the 0.25, 0.80, and 2.50 mg CPFB/L groups, respectively. The female relative liver weight increases were 12, 18, and 71% in the respective exposure groups. The heart/body weight ratios of male test mice were all lower than controls, although the difference was significant only in the group exposed to the highest concentration.

TABLE 13. ORGAN WEIGHTS[a] AND ORGAN TO BODY WEIGHT RATIOS (%) OF MALE B6C3F1 MICE FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

	Control	0.25 mg/L	0.80 mg/L	2.50 mg/L
Kidney	0.51 ± 0.02	0.53 ± 0.01	0.49 ± 0.02	0.50 ± 0.02
Ratio[b]	1.83 ± 0.05	1.92 ± 0.03	1.78 ± 0.06	1.88 ± 0.07
Heart	0.19 ± 0.01	0.16 ± 0.01	0.15 ± 0.01[c]	0.15 ± 0.01[d]
Ratio	0.69 ± 0.03	0.58 ± 0.05	0.56 ± 0.03	0.55 ± 0.02[c]
Brain	0.49 ± 0.01	0.50 ± 0.01	0.47 ± 0.01	0.51 ± 0.02
Ratio	1.78 ± 0.04	1.82 ± 0.03	1.72 ± 0.06	1.90 ± 0.07
Liver	1.42 ± 0.09	1.63 ± 0.08	1.63 ± 0.06	1.85 ± 0.08[c]
Ratio	5.13 ± 0.26	5.93 ± 0.24	5.97 ± 0.24	6.96 ± 0.32[d]
Spleen	0.09 ± 0.01	0.10 ± 0.00	0.10 ± 0.01	0.08 ± <0.01
Ratio	0.31 ± 0.02	0.36 ± 0.02	0.35 ± 0.02	0.31 ± 0.01
Lungs	0.32 ± 0.04	0.30 ± 0.02	0.30 ± 0.02	0.32 ± 0.03
Ratio	1.15 ± 0.12	1.07 ± 0.08	1.10 ± 0.07	1.21 ± 0.14
Adrenal	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01
Ratio	0.05 ± <0.01	0.05 ± <0.01	0.04 ± 0.01	0.05 ± 0.01
Testes	0.24 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.22 ± 0.02
Ratio	0.87 ± 0.02	0.89 ± 0.04	0.80 ± 0.04	0.83 ± 0.05
Epididymis	0.15 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Ratio	0.53 ± 0.04	0.54 ± 0.05	0.63 ± 0.04	0.59 ± 0.04
Whole Body	27.66 ± 0.48	27.38 ± 0.22	27.38 ± 0.33	26.62 ± 0.46

[a] Mean ± S E M , N = 6

[b] Organ weight/body weight x 100

[c] Significantly different from control at $p<0.05$ using a two-factorial analysis of variance corrected for multiple comparisons

[d] Significantly different from control at $p<0.01$ using a two-factorial analysis of variance corrected for multiple comparisons

TABLE 14. ORGAN WEIGHTS[a] AND ORGAN TO BODY WEIGHT RATIOS (%) OF FEMALE B6C3F1 MICE FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

	Control	0.25 mg/L	0.80 mg/L	2.50 mg/L
Kidney	0.38 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.41 ± 0.01
Ratio[b]	1.59 ± 0.02	1.53 ± 0.04	1.46 ± 0.02	1.70 ± 0.06
Heart	0.12 ± 0.01	0.12 ± <0.01	0.13 ± 0.01	0.14 ± 0.01
Ratio	0.53 ± 0.02	0.52 ± 0.01	0.56 ± 0.03	0.56 ± 0.02
Brain	0.51 ± 0.02	0.50 ± 0.01	0.51 ± <0.01	0.45 ± 0.05
Ratio	2.14 ± 0.06	2.16 ± 0.08	2.15 ± 0.02	1.87 ± 0.21
Liver	1.10 ± 0.04	1.22 ± 0.05[c]	1.32 ± 0.03[c]	1.86 ± 0.08[c]
Ratio	4.67 ± 0.06	5.24 ± 0.14	5.52 ± 0.12	7.98 ± 0.25[d]
Spleen	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Ratio	0.33 ± 0.01	0.36 ± 0.02	0.35 ± 0.03	0.32 ± 0.03
Lungs	0.31 ± 0.03	0.28 ± 0.02	0.23 ± 0.02	0.26 ± 0.02
Ratio	1.33 ± 0.16	1.19 ± 0.07	0.96 ± 0.08	1.10 ± 0.08
Adrenal	0.01 ± <0.01	0.02 ± <0.01	0.02 ± <0.01	0.02 ± <0.01
Ratio	0.06 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.08 ± 0.02
Ovary	0.03 ± <0.01	0.03 ± <0.01	0.04 ± <0.01	0.03 ± <0.01
Ratio	0.11 ± 0.02	0.13 ± 0.02	0.18 ± 0.04	0.12 ± 0.02
Uterus	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.02	0.14 ± 0.02
Ratio	0.54 ± 0.04	0.64 ± 0.04	0.65 ± 0.10	0.63 ± 0.08
Vagina	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.10 ± 0.02
Ratio	0.44 ± 0.04	0.45 ± 0.05	0.52 ± 0.06	0.41 ± 0.08
Whole Body	23.68 ± 0.85	23.30 ± 0.56	23.83 ± 0.31	23.52 ± 0.58

[a] Mean ± S.E.M., N = 6 except 2.50 mg/L, N = 5

[b] Organ weight/body weight x 100

[c] Significantly different from control at $p < 0.05$ using a two-factorial analysis of variance corrected for multiple comparisons

[d] Significantly different from control at $p < 0.01$ using a two-factorial analysis of variance corrected for multiple comparisons

Microscopic changes noted in F-344 rats that were attributable to exposure were limited to the livers of female rats and the kidneys of male rats (**Table 15**). Necrosis of individual hepatocytes was a consistent finding in the livers of CPFB-exposed female rats. Affected hepatocytes were characterized by pyknotic or karyorrhectic nuclei and increased cytoplasmic eosinophilia. In some instances necrotic hepatocytes were associated with variable numbers of mononuclear inflammatory cells, mostly macrophages and lymphocytes. In other areas, affected hepatocytes did not elicit such a cellular reaction. There was not a consistent pattern to the location of necrotic hepatocytes within the hepatic lobule. Although liver weights of CPFB-exposed male and female rats were significantly elevated at the conclusion of the study, there were no morphologic differences evident between control and test rats at the light microscopic level. The formation of hyaline droplets was a consistent finding in the proximal convoluted tubules of the kidneys of all CPFB-exposed male rats.

Microscopic lesions noted in B6C3F1 mice that were attributable to exposure were limited to the liver (**Table 16**). In both male and female 2.50 mg CPFB/L-exposed mice, hepatocytes in the midzonal and, to a lesser degree, centrilobular regions of most hepatic lobules exhibited mild hepatocytomegaly characterized by increased amounts of finely granular eosinophilic cytoplasm. The enlarged hepatocytes tended to distort hepatic plate architecture with compression of sinusoidal spaces. The microscopic hepatocytomegaly correlates well (male mice $R = 0.58$, $p < 0.0028$; female mice $R = 0.89$, $p < 0.0001$) with the statistically significant ($p < 0.01$) increase in relative liver weights in the high-concentration group. Hepatocytomegaly and a corresponding increase in relative liver weight were not evident in the mid- or low-concentration CPFB-exposed groups.

Another treatment-related change in the livers of male and female mice was necrosis of individual hepatocytes. This lesion was present in all CPFB-exposed groups with approximately the same degree of severity. This lesion was similar morphologically to the liver changes described in female rats. Single cell necrosis was evident in control group livers, but at a reduced incidence. Other microscopic changes noted in other organs were not treatment related, but were spontaneously occurring, age related changes.

TABLE 15. SUMMARY OF SELECTED MICROSCOPIC LESIONS OBSERVED IN F-344 RATS FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

Organ-Lesion	Incidence (%)				Severity[a]			
	0.0 mg/L	0.25 mg/L	0.80 mg/L	2.50 mg/L	0.0 mg/L	0.25 mg/L	0.80 mg/L	2.50 mg/L
Liver –Single Cell Necrosis								
Male	0	0	0	0	0.0	0.0	0.0	0.0
Female	0	80[b]	90[b]	60[b]	0.0	0.8[b]	1.0[b]	0.6[b]
Kidney –Tubular Mineralization								
Male	0	0	0	0	0.0	0.0	0.0	0.0
Female	50	0	0	90	0.5	0.0	0.0	0.9[c]
–Hyaline Droplets								
Male	0	100[b]	100[b]	100[b]	0.0	1.0[b]	1.1[b]	1.5[b]
Female	0	0	0	0	0.0	0.0	0.0	0.0

[a] Severity scoring system defined as: 0 = no lesion, 1 = minor or very slight, 2 = slight; 3 = moderate, 4 = marked, 5 = severe Groups scores calculated by dividing the sum of individual scores by the number of affected animals

[b] Significantly different from control, $p<0.01$ using Fisher's Exact Test and Yates' Corrected Chi-Square Test

[c] Significantly different from control, $p<0.05$ using Fisher's Exact Test and Yates' Corrected Chi-Square Test

TABLE 16. SUMMARY OF SELECTED MICROSCOPIC LESIONS OBSERVED IN B6C3F1 MICE FOLLOWING 21-DAY REPEATED INHALATION EXPOSURE TO CHLOROPENTAFLUOROBENZENE

Organ-Lesion	Incidence (%)				Severity[a]			
	0.0 mg/L	0.25 mg/L	0.80 mg/L	2.50 mg/L	0.0 mg/L	0.25 mg/L	0.80 mg/L	2.50 mg/L
Liver – Single Cell Necrosis								
Male	17	67	83[b]	100[b]	0.2	0.7[b]	0.8[b]	1.0[b]
Female	50	100[b]	100[b]	83[b]	0.5	1.0[b]	1.0[b]	0.8[b]
– Hepatocytomegaly								
Male	0	0	17	100[b]	0.0	0.0	0.2	1.8[b]
Female	0	0	0	100[b]	0.0	0.0	0.0	2.0[b]

[a] Severity scoring system defined as: 0 = no lesion; 1 = minor or very slight, 2 = slight; 3 = moderate; 4 = marked, 5 = severe Groups scores calculated by dividing the sum of individual scores by the number of affected animals

[b] Significantly different from control, $p<0.01$ using Fisher's Exact Test and Yates' Corrected Chi-Square Test

SECTION 5

GENOTOXICITY

The SCE/cell, the H value (SCE variance/SCE mean), the AGT, and the MI data from male and female B6C3F1 mice exposed to CPFB are presented in **Table 17**. A two-way analysis of variance of the data from male and female mice revealed the absence of a significant difference in SCE ($p = 0.0693$) response between the sexes. However, a significant sex by dose interaction was observed for AGT data ($p = 0.0417$), thereby requiring the separate statistical analysis of male and female data. Exposure to CPFB at concentrations of 0.25, 0.80, and 2.50 mg/L for three weeks (6 h per day, excluding weekends) did not indi ce an increase in the frequency of SCE in the bone marrow of male and female mice. Similarly, a di_persion analysis of the SCE data also indicated a lack of damage induced by CPFB. In the bone marrow the rate of cellular proliferation was not altered in either male or female mice, but the number of proliferating cells was significantly increased ($p = 0.0010$). As expected, a significant increase in SCE frequency was detected in the bone marrow of positive control mice injected with DMBA ($p < 0.0001$).

Data collected from scoring peripheral blood smears are presented in **Table 18**. Because of a significant sex difference in MN-NCE numbers at day 0 ($p = 0.0174$), and in MN-PCE numbers at day 9 ($p = 0.0020$) and at day 21 ($p = 0.0002$), MN data were analyzed without pooling between the sexes. For male mice, at every sample time, exposure to CPFB failed to induce an increase in the number of MN-PCE or in the number of MN-NCE. For female mice, MN-NCE frequencies were not significantly elevated at any sample time, while the number of MN-PCE was significantly increased at day 16 only ($p = 0.0038$). Because none of the micronucleated erythrocytes in any of the control or CPFB-exposed mice contained more than one MN, an evaluation of the distribution of MN among cells was not conducted. Significant increase in MN-PCE and MN-NCE populations was detected in the bone marrow of mice used as a positive control ($p < 0.0001$). After pooling %PCE data between male and female mice ($p > 0.12$), a trend test analysis of these data indicated a significant depression in the rate of erythropoiesis at days 9 ($p = 0.0258$) and 21 ($p = 0.0078$).

TABLE 17. GROUP BONE MARROW CYTOGENETIC DATA FOR B6C3F1 MICE EXPOSED TO CPFB

Concent. (mg/L)	Sex	SCE[a] Mean	±	S.E.M.	N	H[b] Stat. Mean	±	S.E.M.	N	AGT[c] (h) Mean	±	S.E.M.	N	MI[d] (%) Mean	±	S.E.M.	N
0	M	5.27	±	1.079	4	1.18	±	0.110	4	14.24	±	0.935	6	2.70	±	0.425	6
	F	7.26	±	1.040	4	1.80	±	0.556	4	14.75	±	0.609	6	2.68	±	0.311	6
	M + F	6.26	±	0.789	8	1.49	±	0.287	8					2.69	±	0.251	12
0.25	M	4.11	±	0.111	4	0.85	±	0.129	4	14.72	±	1.374	6	2.72	±	0.334	6
	F	5.87	±	0.804	4	1.53	±	0.253	4	16.74	±	0.804	6	1.87	±	0.431	6
	M + F	4.99	±	0.502	8	1.19	±	0.184	8					2.29	±	0.290	12
0.80	M	5.95	±	0.454	4	1.31	±	0.183	4	14.64	±	0.920	6	2.43	±	0.612	6
	F	5.79	±	0.440	4	1.48	±	0.381	4	16.64	±	0.750	6	3.10	±	0.509	6
	M + F	5.87	±	0.294	8	1.39	±	0.198	8					2.77	±	0.393	12
2.50	M	4.69	±	0.143	4	1.44	±	0.136	4	16.48	±	0.968	6	4.13	±	0.313	6
	F	4.88	±	0.516	4	1.07	±	0.056	4	13.68	±	0.772	5	3.58	±	0.477	5
	M + F	4.78	±	0.250	8	1.26	±	0.098	8					3.82	±	0.258[e]	11
DMBA positive control data (mg/kg)																	
0	M	5.25	±	0.310	4	0.93	±	0.177	4	12.84	±	0.144	4	2.75	±	0.343	4
2.5	M	16.40	±	1.315[f]	4	3.13	±	1.180[f]	4	13.18	±	0.211	4	3.95	±	0.378	4

[a]SCE = Sister chromatid exchange/cell frequency based on 25 cells per mouse
[b]H = SCE variance/SCE mean
[c]AGT = Average generation time in hours based on 100 metaphase cells per mouse
AGT = BrdUrd exposure time/1 x frequency of M1 + 2 x frequency of M2 + 3 x freqency of M3 metaphase cells
[d]MI = Mitotic index based on scoring 1000 nucleated cells per mouse
[e]Significantly different at alpha = 0.05 using a one-tailed Student's t test, based on separate group variances
[f]Significantly different at alpha = 0.05 using a one-tailed trend t test, based on individual mouse data

TABLE 18. GROUP PERIPHERAL BLOOD MICRONUCLEUS DATA FOR B6C3F1 MICE EXPOSED TO CPFB

MN-PCE[a]

Exposure Duration (days)	Sex	Dose = 0			Dose = 0.25			Dose = 0.80			Dose = 2.50			Positive Control		
		Mean	S.E.M.	N[b]	Mean	S.E.M.	N	Mean	S.E.M.	N	Mean	S.E.M.	N	Mean	S.E.M.	N
0	M	2.33	± 0.667	6	2.00	± 0.516	6	3.33	± 0.843	6	2.67	± 0.955	6	28.00	± 4.412	6[c]
	F	1.17	± 0.307	6	2.67	± 0.760	6	2.67	± 0.494	6	2.33	± 0.422	6			
9	M	4.33	± 0.333	6	2.00	± 0.577	6	2.83	± 0.477	6	2.17	± 0.401	6	25.33	± 3.528	6[c]
	F	1.17	± 0.601	6	2.67	± 0.803	6	1.17	± 0.477	6	1.33	± 0.333	6			
16	M	3.17	± 0.601	6	3.33	± 0.174	6	3.33	± 1.116	6	2.50	± 0.563	6	27.50	± 3.845	6[c]
	F	2.00	± 0.683	6	1.83	± 0.543	6	2.00	± 0.516	6	4.00	± 0.516	6[c]			
21	M	3.00	± 0.365	6	3.67	± 0.494	6	2.00	± 0.258	6	2.83	± 0.477	6	28.00	± 2.745	6[c]
	F	1.50	± 0.428	6	1.67	± 0.422	6	1.50	± 0.563	6	1.80	± 0.342	5			

[a]MN-PCE = Micronucleated polychromatic erythrocytes

[b]Group mean values are of micronucleated erythrocytes per 1000 erythrocytes ± the standard error of the mean among N animals

[c]Significantly different from concurrent control data at α = 0.05 based on a one-tailed Pearson chi-square test, based on pooled mouse data

[d]Significantly different from concurrent control data at α = 0.05 based on a one-tailed trend test, based on pooled MN data

TABLE 18. CONTINUED

MN-NCE[a]

Exposure Duration (days)	Sex	Dose = 0			Dose = 0.25			Dose = 0.80			Dose = 2.50			Positive Control		
		Mean	S.E.M.	N[b]	Mean	S.E.M.	N	Mean	S.E.M.	N	Mean	S.E.M.	N	Mean	S.E.M.	N
0	M	2.83	± 0.601	6	2.17	± 0.477	6	2.50	± 0671	6	1.67	± 0.333	6	8.33	± 1.022	6[c]
	F	1.67	± 0.615	6	1.83	± 0.477	6	1.17	± 0.543	6	0.83	± 0.307	6			
9	M	2.00	± 0.516	6	1.50	± 0.428	6	1.50	± 0.563	6	0.83	± 0.477	6	9.33	± 1.382	6[c]
	F	1.83	± 0.477	6	2.33	± 0.494	6	2.17	± 0.980	6	1.17	± 0.167	6			
16	M	2.67	± 0.919	6	1.17	± 0.307	6	2.17	± 0.477	6	2.33	± 0.760	6	11.33	± 1.022	6[c]
	F	2.50	± 0.719	6	1.67	± 0.333	6	2.00	± 0.516	6	1.67	± 0.422	6			
21	M	1.17	± 0.401	6	1.33	± 0.422	6	1.83	± 0.654	6	1.67	± 0.333	6	10.33	± 1.856	6[c]
	F	1.83	± 0.703	6	1.67	± 0.558	6	2.17	± 0.654	6	1.60	± 0.228	5			

[a]MN-PCE = Micronucleated polychromatic erythrocytes

[b]Group mean values are of micronucleated erythrocytes per 1000 erythrocytes ± the standard error of the mean among N animals

[c]Significantly different from concurrent control data at $\alpha = 0.05$ based on a one-tailed Pearson chi-square test, based on pooled mouse data

[d]Significantly different from concurrent control data at $\alpha = 0.05$ based on a one-tailed trend test, based on pooled MN data

TABLE 18. CONTINUED

%PCE[a]

Exposure Duration (days)	Sex	Dose = 0 Mean	S.E.M.	N[b]	Dose = 0.25 Mean	S.E.M.	N	Dose = 0.80 Mean	S.E.M.	N	Dose = 2.50 Mean	S.E.M.	N	Positive Control Mean	S.E.M.	N
0	M	2.63 ±	0.762	6	2.53 ±	0.355	6	2.88 ±	0.494	6	2.25 ±	0.402	6	2.12 ±	0.381	6[c]
	F	2.60 ±	0.392	6	2.07 ±	0.409	6	3.28 ±	0.356	6	2.62 ±	0.335	6			
	M + F	2.62 ±	0.409	12	2.30 ±	0.267	12	3.08 ±	0.296	12	2.43 ±	0.256	12			
9	M	2.18 ±	0.180	6	2.13 ±	0.178	6	2.32 ±	0.375	6	1.82 ±	0.202	6	2.07 ±	0.397	6[c]
	F	2.63 ±	0.216	6	2.58 ±	0.257	6	2.25 ±	0.226	6	2.03 ±	0.199	6			
	M + F	2.41 ±	0.150	12	2.36 ±	0.164	12	2.28 ±	0.209	12	1.92 ±	0.139	12[d]			
16	M	3.53 ±	0.312	6	2.77 ±	0.256	6	5.30 ±	2.602	6	2.20 ±	0.167	6	2.02 ±	0.196	6[c]
	F	2.10 ±	0.173	6	3.35 ±	0.385	6	2.05 ±	0.295	6	2.27 ±	0.167	6			
	M + F	2.82 ±	0.375	12	3.06 ±	0.238	12	3.68 ±	1.341	12	2.23 ±	0.113	12			
21	M	2.03 ±	0.194	6	2.70 ±	0.235	6	1.82 ±	0.180	6	1.75 ±	0.184	6	2.32 ±	0.280	6[c]
	F	1.62 ±	0.168	6	2.50 ±	0.257	6	2.03 ±	0.291	6	1.20 ±	0.198	5			
	M + F	1.82 ±	0.138	12	2.60 ±	0.169	12	1.92 ±	0.166	12	1.50 ±	0.159	11[d]			

[a]%PCE = Percentage of polychromatic erythrocytes

[b]Group mean values are of micronucleated erythrocytes per 1000 erythrocytes ± the standard error of the mean among N animals

[c]Significantly different from concurrent control data at α = 0.05 based on a one-tailed Pearson Chi-square test, based on pooled mouse data

[d]Significantly different from concurrent control data at α = 0.05 based on a one-tailed trend test, based on individual mouse data

SECTION 6

DISCUSSION

Of prime importance was the toxic effect of CPFB on the liver of mice exposed at the highest concentration. The morphologic results documented gross liver hypertrophy and microscopic hepatocytomegaly as the principal manifestations of CPFB-induced hepatotoxicity. The changes observed in alkaline phosphatase activity and liver weight ratios of exposed female rats were considered directly related to CPFB exposure because there was significant morphologic alteration in the hepatocytes. Although hyaline droplet accumulation in proximal tubules was prevalent in all exposed male rat kidneys, the severity of the lesion was minimal and renal epithelial necrosis or other morphometric indicators of renal disease were not present. The decrease in MCV had no physiclogical significance since the RBC and the hematocrit used to compute MCV were within normal ranges. Although the BUN and total protein values of the high concentration rats were different from controls, the differences were not considered physiologically significant because all values were within normal ranges reported by other investigators (Ringler and Dabich, 1979.) The basis for the decrease in SGOT values with increasing exposure concentrations has not been determined. Since SGOT is an enzyme that increases when there is muscle damage, liver cell injury, or reduced glomerular filtration, the changes in this enzyme are probably not indicative of a disease process.

Exposure of male and female B6C3F1 mice to CPFB, 6 h per day for three weeks (excluding weekends), failed to induce an increase in SCE (based on the mean or on the intercellular dispersion). However, in female mice only a slight, but significant, increase in MN-PCE frequency was detected at a single sample time (day 16). Although a repeat study is required to verify the accuracy of this finding, the weakness of the response combined with the lack of supporting data at day 9 and/or day 16 suggested that the response was spurious. The absence of a significant increase in SCE and the nature of the MN response indicated that an evaluation of chromosomal aberration frequencies in bone marrow cells was unwarranted. Also, exposure to CPFB did not result in an altered rate of cellular proliferation in bone marrow but did depress the rate of erythropoiesis at days 9 and 21 and increased the MI in mice sampled at the end of the exposure period. These altered responses indicated that exposure to CPFB resulted in signs of systemic stress in mice. The absence of significant and internally reproducible genotoxic damage in bone marrow of mice suggested that CPFB is not genotoxic.

SECTION 7

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QUALITY ASSURANCE

The study, "Evaluation of the Potential of Inhaled Chloropentafluorobenzene to Induce Toxicity in F-344 Rats and B6C3F1 Mice and Sister Chromatid Exchanges and Micronuclei Formation in B6C3F1 Mice," was conducted by the NSI Technology Services Corporation, Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practices Guidelines, 40CFR PART 792. The various phases of this study were inspected by members of the Quality Assurance Unit. Results of these inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION:	ITEM INSPECTED:
26, 27JUL88	Target Organ Toxicity rat pre-exposure weighing, randomization, dose group assignment, inhalation study initiation.
14JUL88	Genotoxicity mouse inhalation exposure initiation.
22, 29JUL, 3AUG88	Genotoxicity mouse bleeding, weighing.
3AUG88	Genotoxicity mouse implant surgical procedure.
4AUG88	Genotoxicity mouse sacrifice, organ weights.
8SEP88	Clinical Pathology procedures.
14FEB89	Histopathology specimens.
19JUN-21JUL89	Final Report audit.

The Quality Assurance Unit has determined by review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretation presented in this Final Report. There were, however, discrepancies noted during inspections which would preclude general acceptance of this study as having been conducted according to the strict requirements as outlined in EPA TITLE 40 Part 792.

M. G. Schneider
QA Coordinator
Toxic Hazards Research Unit
Date 26 July 1989